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(57) Abstract

Method of contraception utilizing IL-1 receptor antagonists are disclosed. In one embodiment, the method includes preventing ovulation and implantation of an embryo by administering to a subject a pharmacologically effective dose of an interleukin-1 (IL-1) receptor antagonist during the pre-implantation period. In a related method, the IL-1 receptor antagonist dislodges the implanted embryo, when it is administered during the post-implantation period. Also disclosed are contraceptive compositions, including IL-1 receptor antagonists, for use in the methods of the invention. Further disclosed is a method of selecting compounds for use in the contraceptive methods of the invention.

METHOD OF CONTRACEPTION

Field of the Invention

The present invention relates to methods and devices interfering with implantation of embryonic stage organisms in the maternal endometrium, and for preventing ovulation.

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Background of the Invention

The search for convenient and reliable means of birth control is one which occupies a significant portion of the U.S. pharmaceutical industry. Public reluctance to accept abortion as a means of eliminating unwanted pregnancies has resulted in intensified efforts to provide alternative methods of post-coital birth control.

Currently the most widely used methods of birth control are those which prevent fertilization of the ovum. Known preventive methods currently in use include those which prevent conception by physical means (condoms), combined physical and chemical barriers (e.g., diaphragms and spermicides) or estrogen-based contraceptive formulations. Prevention of pregnancy may also be effected by

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insertion in the uterus of an intrauterine device (IUD), which may include a chemical component, such as the hormone progesterone. Although the foregoing methods of birth control are generally reliable when used correctly, they suffer from the disadvantage that a certain amount of planning must be taken on the part of at least one of the sexual partners prior to sexual intercourse.

As stated above, public attitudes in the U.S. have deterred widespread use of post-coital forms of birth control, or abortifactants. Mifepristone (RU-486) is an antiprogestin which, when given during the first trimester of pregnancy, stimulates release of prostaglandins in the uterus, stimulates uterine motility and effects detachment from the uterus to cause abortion of the fetus. This compound, though an effective abortifactant, causes severe discomfort and may induce uterine bleeding requiring further medical management. Moreover, RU-486 it has not yet been approved for medical use in the United States.

Currently, there is only one formulation that is approved for prevention of pregnancy in those women who have had unprotected sexual intercourse. Diethylstilbestrol, the so-called "morning after pill", is a synthetic nonsteroidal compound having estrogen agonistic properties. This compound has the disadvantage that if gestation occurs, teratogenic effects such as vaginal adenocarcinoma and uterine malformations may occur in female fetuses. Also, a significant incidence of nausea and vomiting leads some patients to discontinue the medication in the middle of therapy.

Summary of the Invention

In one aspect, the invention includes a composition for use in preventing ovulation and

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implantation of an embryo in a uterus of a mammalian subject, comprising an IL-1 receptor antagonist.

The interleukin-1 receptor antagonist may be a peptide, such as interleukin receptor antagonist IL-1ra (SEQ ID NO: 2) or icIL-1ra (SEQ ID NO: 4), or an oligopeptide selected from a combinatorial library. In the latter case, the compound is selected from a combinatorial library of compounds, by the steps of measuring (i) agonist activity, and (ii) antagonist activity of a library of compounds, in a functional IL-1 receptor assay, and selecting a specific compound in the library if it has (i) substantially no agonist activity, and (ii) substantial antagonist activity.

Alternatively, as compound may be selected by (i) reacting compounds from a library with cells having an IL-1 cell-surface receptor, in the presence of a reporter-labeled IL-1, (ii) assaying the ability of library compounds to displace labeled IL-1 from such cell-surface receptors, and (iii) selecting a compound from the library effective to displace labeled IL-1 from the cells.

Also forming part of the invention is a contraceptive device for use in preventing ovulation and implantation of an embryo or in dislodging an embryo in a mammalian uterus. The device includes a vaginal insert or a cervical cap, and compound release means in said insert or cap for releasing an IL-1 receptor antagonist compound at a dose effective to prevent or disrupt implantation of an embryo in the uterus.

In another aspect, the invention includes a method of identifying a compound for use in preventing ovulation and implantation of an embryo or in dislodging an embryo in a mammalian uterus. The method includes measuring (i) agonist activity, and (ii) antagonist activity of a library of

compounds, in a functional IL-1 receptor assay, and selecting a specific compound in the library if it has (i) substantially no agonist activity, and (ii) substantial antagonist activity.

5   The method may further include measuring further includes measuring the binding affinity of the compound in an IL-1R tI IL-1 ligand competitive displacement binding assay, and said selecting further includes selecting the compound if its

10   affinity in such binding assay is at least within two orders of magnitude of the binding affinity of an IL-1 receptor antagonist selected from the group consisting of IL-1ra and icIL-1ra.

      IL-receptor antagonist compounds used in the

15   methods and compositions described herein have several advantages over known contraceptive methods. They act locally, rather than systemically, resulting in few if any systemic side effects. Moreover, they are effective after the fertilization

20   event and can be used as an alternative to currently practiced post-fertilization means of terminating pregnancy.

Brief Description of the Figures

25   Figures 1A and 1B show Northern blots (1A) of IL-1R tI mRNA in cultured human endometrial stromal cells (ESC), where the migration positions of 18S and 28S ribosomal RNA markers are indicated to the left, and corresponding quantitation of the blots by

30   densitometric analysis (1B);

      Figures 2A and 2B show Northern blots (2A) of IL-1R TI MRNA in cultured human endometrial epithelial cells (EEC), where marker positions are indicated as in Figure 1A, and show corresponding

35   quantitation of the blots by densitometric analysis (2B);

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Figure 3 (A-E) shows indirect immunofluorescence immunolocalization of mouse IL-1R TI in mouse uterus (3A-3D) and of IL-1β (3E) in the mouse embryo;

Figure 4 (A-E) shows micrographs of sections of mouse uteri taken at various post-fertilization stages of untreated mice (4A, 4C, 4E) and of mice treated with hr IL-1ra (4B, 4D, 4F);

Figure 5 is a temporal depiction of the human female menstrual cycle, indicating appropriate times in the cycle at which intervention with IL-1ra receptor blockade will prevent embryonic implantation; and

Figure 6 shows DNA and amino acid sequences of IL-1ra as SEQ ID NO: 1 and SEQ ID NO: 2, respectively; and

Figure 7 shows the DNA and amino acid sequences of iCIL-1ra as SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

Detailed Description of the Invention

I. Definitions

The term "endometrium", as used herein refers to the glandular inner layer of the uterus, overlying a muscular myometrial layer.

The term "endometrial stromal cell" (abbreviated "ESC") refers to a specific endometrial cellular component underlying epithelial cells in the uterus.

The term "endometrial epithelial cells" (abbreviated "EEC"), refers to a specific cellular component of the uterine endometrium. These cells are in contact with the lumen of the uterus.

The term "oocyte" refers to the immature female egg cell present in the ovaries and having a full diploid complement of chromosomes.

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The term "ovum" refers to the mature female haploid egg cell which has matured from an oocyte by undergoing meiosis. The ovum is released at the time of ovulation from the follicles of the ovaries.

5   The term "ovulation" refers to the release by the follicles of one or more ova. Ovulation is characterized in humans by specific hormonal changes; in particular, it occurs just subsequent to a spike in luteinizing hormone (LH) and follicle

10   stimulating hormone (FSH) levels and a decrease in level of estrogen.

The term "embryo" refers to a fertilized ovum.

The term "blastocyst" refers to a specific stage of embryo, characterized by the presence of a

15   central cavity surrounded by dividing cells.

The term "peri-implantation" period, refers to a time period shortly before, during and after uterine implantation of the embryo, as described in Section V herein.

20   The term "IL-1 receptor antagonist" refers to a compound that blocks the effects of endogenous or exogenously added IL-1. IL-1 receptor antagonists may act competitively or non-competitively at the receptor level.

25   The term "IL-1 inhibitor" refers to a compound that blocks IL-1 activity. IL-1 inhibitors include IL-1 receptor antagonists, but also include agents that act by decreasing an amount of endogenous IL-1 available or released.

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II.   Interleukin-1 Receptor Antagonists

Interleukin-1 receptor antagonist compounds are characterized by an ability to inhibit binding and biological effects of IL-1α and IL-1β, as described

35   in Section III, below. Receptor antagonist compounds include, in addition to competitive inhibitors   of   receptor   binding,   other

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macromolecules, such as antibodies or exogenously added IL-1 binding proteins that interfere with IL-1 binding to its receptor.

IL-1 receptor antagonist compounds that are particularly useful in practicing the present invention are those which interfere with the interaction between IL-1 and its receptor(s) in the uterus. Particularly useful compounds include IL-1ra, a specific peptide antagonist and derivatives thereof, collectively termed IL-1ra peptides. It is the discovery of the present invention that blocking the interaction between embryonic IL-1 and uterine IL-1 receptors, particularly type I receptors, in the mammalian uterus results in disruption of implantation of the embryo in the uterus.

A.   IL-1 Receptor Antagonists

Several naturally occurring IL-1 receptor antagonist compounds have been identified and isolated from biological sources including serum, synovial exudates, and urine. Some of these compounds are not specific to the IL-1 receptor, since they also inhibit binding and/or activity of other cytokines, notably IL-2. While such non-specific inhibitors may be used in practicing the methods of the invention described herein, antagonist compounds that are specific to the IL-1 receptor, and particularly the IL-1 type I receptor (IL-1R TI) are generally preferred for use in the invention.

B.   IL-1ra peptides

IL-1ra is IL-1 receptor antagonist that can be used in practicing the invention. IL-1ra is a 22-25 kDA peptide which is found in the urine of febrile human patients suffering from monocytic leukemia (Seckinger), but has also been identified in other tissues, including human monocytes (Eisenberg).

10

This compound is an antagonist of binding of IL-1α and IL-1β at the IL-1 receptor, particularly at the IL-1 Type I receptor.

As is described in Section III, below, IL-1ra selectively inhibits binding of IL-1 (α or β) to the IL-1 receptor. The selectivity of this compound is evidenced by the fact that it does not inhibit biological effects of IL-2 or other cytokines.

IL-1ra cDNA has been identified and cloned from a monocyte library (Eisenberg). The complete DNA sequence is shown in Figure 7 as SEQ ID NO: 1. Based on the cDNA sequence, an amino acid sequence has been deduced and verified. This sequence is shown in Figure 7 as SEQ ID NO: 2. The cDNA shown encodes a 25 amino acid secretory peptide leader sequence followed by a 152 amino acid polypeptide.

For use in the present invention, IL-1ra is generally isolated from natural sources or produced by recombinant methods known in the art (Seckinger; Ausubel) and described in the sections which follow.

Derived from the same gene, but having an alternate splicing pattern, is an intracellular form of IL-1ra, termed icIL-1ra (Haskill). This IL-1ra peptide lacks the N-terminal 21 amino acids the 25 residue signal sequence of IL-1ra. In their place are three different amino acids. The DNA and peptide sequences are shown as SEQ ID NO: 3 and SEQ ID NO: 4, respectively, in Figure 7.

1. Isolation of IL-1ra peptides

    a.   Purification of IL-1ra from Biological Sources.

IL-1ra has been isolated from a number of biological sources, using standard biochemical purification procedures known in the art. One such procedure that has been published in the medical literature by Carter, et al. involves isolation of the peptide from culture supernatants of

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differentiated U937 (myelomonocytic) cells. This
method includes ultrafiltration through molecular
weight cut-off filters, Superose 12 FPLC, followed
by two rounds of TSK Biosil 125 HPLC and C4 RP HPLC.
5   (Carter). This method, summarized in Example 6) can
be used to obtain IL-1ra of sufficient purity for
use in the inventive methods described herein.
Alternatively, the purification method described by
Hannum et al., incorporated herein by reference, can
10   be used to purify IL-1ra for use in the methods and
compositions of the present invention.

              b .   Recombinant Production of IL-1ra.
         Human recombinant IL-1ra (hr IL-1ra) can be
15   expressed in bacterial or mammalian expression
systems, according to methods known in the art. In
one such method, used to produce IL-1ra for the
studies reported herein, and detailed in Example 5,
IL-1ra is expressed as a fusion protein using a pMAL
20   vector (New England Biolabs, Beverly, MA). This
vector is constructed to contain the DNA clone shown
as SEQ ID NO: 1 in Figure 6. Alternatively or in
addition, the method described by Hannum et al. in
U.S. Patent No. 5,075,222, incorporated herein by
25   reference, is used to produce IL-1ra
         The expressed fusion protein is purified from
the periplasm of _E. coli_ by Amylose affinity
chromatography (New England Biolabs). The isolated
fusion protein is cleaved by Factor Xa treatment to
30   produce hr IL-1ra and the carrier maltose binding
protein. Homogeneity of hIL-1ra is achieved with
further purification using, for example, Q-sepharose
and Sephacryl S-300 gel filtration chromatographies
(Pharmacia), according to methods known in the art.
35   The purified protein has a molecular mass (Mr) of
17,000. Specific activity of recombinant IL-1ra can
measured by any of a number of biological assays for

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ability to block IL-1 activity.   One such assay is
a mouse D10 cell proliferation assay (Polan, *et
al.*), detailed in Example 8 and described in Section
III below.

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2.   Isolation of icIL-1ra

The intracellular form of IL-1ra (icIL-1ra) is
produced by recombinant expression methods, such as
the COS cell expression method described in the
10   reference by Haskill, et al. (incorporated herein by
reference). According to this method, COS cells are
transfected with a SR/α vector containing a *Pst* I
fragment containing the sequence shown in Figure 7
as SEQ ID NO: 3.   Cell lysates are then used for
15   purification of the icIL-1ra peptide, essentially as
described for IL-1ra, above.

C.   Derivatives of IL-1ra Peptides

In   accordance   with   known   principles   of
20   conservative amino acid substitutions in a protein,
it can be appreciated that derivatives of IL-1ra can
be constructed by making conservative amino acid
substitutions   into   the   parent   IL-1ra   primary
structure SEQ ID NO: 2, or into the parent icIL-1ra
25   structure SEQ ID NO: 4.   Such conservative amino
acid substitutions are known in the art to encompass
such substitutions as serine for alanine and the
like. For example, according to a standard Dayhoff
frequency   exchange   matrix,   twenty   naturally
30   occurring amino acids are placed in six categories,
based   on   substitutions   in   nature   (Schwartz).
Conservative substitutions can be made between amino
acids present in any single class.   According to
this scheme, the six classes are as follows: Class
35   I, CYS; Class II, Ser, Thr, Ala, Gly, representing
small   aliphatic   side   chains   and   OH   group   side
chains; Class III, Asn, Asp, Glu, Gln, representing

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neutral and negatively charged side chains capable
of forming hydrogen bonds; Class IV, His, Arg, Lys,
representing basic polar side chains; Class V, Ile,
Val, Leu, representing branched aliphatic side
5   chains, and Met; Class VI, Phe, Tyr, Trp,
representing aromatic side chains. Proline and 4-
hydroxyproline are members of Class II; however,
substitution of these amino acids for other Class II
residues may introduce new secondary structure into
10   the polypeptide backbone. Proline and 4-
hydroxyproline may be considered to comprise an
additional class, for purposes of conservative
substitution. Furthermore, each class may include
certain related amino acid analogs, such as
15   ornithine, homoarginine, N-methyl lysine, dimethyl
lysine (Class IV) and halogenated tyrosine in Group
VI.

       IL-1ra derivatives suitable for use in the
present invention will have an IL-1 antagonist
20   profile that is substantially like that exhibited by
IL-1ra, as described in Section III, below. That is
such derivatives will have very little or no IL-1
agonist activity and will have activity profiles
that approximate those of IL-1ra. By "approximate"
25   is meant that such compounds will have a potency in
an IL-1 antagonist assay which is minimally within
about 2-3 orders of magnitude of the potency of IL-
1ra or icIL-1ra.

       Thus in selecting as IL-1 receptor antagonist
30   compounds for use in the invention IL-1ra
derivatives as described above, a practitioner will
be guided by the present invention to construct such
derivatives according to the conservative
substitution guidelines discussed above using
35   methods known in the art, such as by site-specific
oligonucleotide-directed mutagenesis techniques
(Ausubel et al.; Maniatis et al.). Available from

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commercial sources are kits for site-directed mutagenesis (Stratagene, La Jolla, CA), that can be used in constructing IL-1ra peptide derivatives.

In accordance with the invention, IL-1ra peptide derivatives formed according to one or more of the above techniques are then tested for binding to the IL-1 receptor (IL-1R t1) and/or for inhibition of IL-1 activity in vitro, according to the methods set forth in Section III, below. Derivative compounds having binding and inhibitory activities, or minimally, functional inhibitory activity, approximating a range defined by the activity of IL-1ra and icIL-1ra, described below, are also utilizable in the methods and compositions of the present invention.

It is also appreciated that in accordance with the discovery of the present invention interference with uterine implantation may also be accomplished by other compounds that diminish the biological effects of IL-1 in vivo, such as by reducing production of IL-1 by the embryo or by stimulating endogenous production of IL-1ra. Thus, a compound such as zymosan (Tenidap®), that reduces secretion of endogenous IL-1, may also find use in the present invention.

D.   Monoclonal antibodies against IL-1 Receptor

Monoclonal antibodies raised against the mouse type I IL-1 receptor have been shown to block immune and inflammatory responses of IL-1 at the receptor (McIntyre). In accordance with the present invention, monoclonal antibodies having such binding and inhibitory properties with respect to the human IL-1R tI, can be used in the methods and compositions of the present invention.

Preferably, monoclonal antibodies will be raised against the human IL-1R tI, according to

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methods well known in the art (Mishell). Immunogen
material for preparing such antibodies will be
prepared by biochemical or recombinant techniques as
described in Section II, then used to immunize a
5   suitable host animal, including, but not restricted
to, mouse, rat, goat, or rabbit. Mice are generally
considered preferable for such purposes.

        Host animals are inoculated with an appropriate
amount of a pure or partially pure human IL-1R tI.
10   The amount will depend on the animal used.   For
mice, an amount of immunogen corresponding to about
1-100 μg protein will be sufficient for such
purposes.   Sera are then tested for presence of
antibodies, such as in an ELISA test according to
15   standard methods known in the art. Spleens are then
removed   from   animals   exhibiting   evidence   of
immunoreactivity with human IL-1R tI.   Dispersed
spleen cells are then fused with an appropriate
fusion   partner   myeloma   cell,   using   hybridoma
20   production   techniques   known   in   the   art   (Harlow).
Cultures   are   further   tested   for   presence   of
immunoglobulin and anti-IL-1R tI activities by ELISA
and/or receptor binding assays, such as the receptor
binding assay described herein.   Preferably, for
25   use in humans, such antibodies will be "humanized"
according   to   recombinant   methods.   For example,
according   to   one   such   method,   mouse-derived
monoclonal antibody variable regions are combined
with human constant regions to produce "humanized"
30   mouse   monoclonal   antibodies   (Taylor).   Such
humanized antibodies may be preferred for use in
humans,   due   to   their   reduced   potential   for
production of non-specific immune reactions.

35   III. Method   of   Screening   for   IL-1   Receptor
           Antagonists
        In accordance with the discoveries described
herein, it can be appreciated that the present

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invention defines a method for selecting or
screening compounds for use as contraceptives. In
this regard, compounds having IL-1 receptor
antagonism activity that is substantially similar to
5   such activity exhibited by IL-1ra will be useful in
the methods and compositions of the invention.

It can be further appreciated that candidate
compounds include, but are not limited to, IL-1ra
peptide derivative compounds, described above.
10   Other preferred candidate compounds include peptide
fragments of IL-1ra, as well peptides and other
compounds generated by combinatorial libraries, as
described below, or a random-sequence peptide
library, such as a library using filamentous phage
15   fUSE5 as a vector (Scott; Cwirla).

A.   Combinatorial Libraries

A variety of combinatorial libraries of random-
sequence oligonucleotides, polypeptides, or
20   synthetic oligomers have been proposed (Kramer;
Houghten, 1985, 1986, 1991, 1992; Ohlmeyer; Dooley,
1993a-1993b; Eichler; Pinella, 1992, 1993; Ecker;
and Barbas). A number of small-molecule libraries
have also been developed (e.g., Ellman).

25   Combinatorial libraries of oligomers may be
formed by a variety of solution-phase or solid-phase
methods in which mixtures of different subunits are
added stepwise to growing oligomers or parent
compound, until a desired oligomer size is reached.
30   A library of increasing complexity can be formed in
this manner, for example, by pooling multiple
choices of reagents with each additional subunit
step (Houghten, 1991).

Alternatively, the library may be formed by
35   solid-phase synthetic methods in which beads
containing different-sequence oligomers that form
the library are alternately mixed and separated,

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with one of a selected number of subunits being added to each group of separated beads at each step (Furka, 1991; Lam, 1991, 1993; Zuckermann; Sebestyen).

5   Still another approach that has been proposed involves the synthesis of a combinatorial library on spatially segregated arrays (Fodor).

In one preferred embodiment, the library is a combinatorial library of hexapeptides or
10   heptapeptides, containing all or some defined subset of permutations of amino acids. The approached generally follows published method (Houghten, 1991).

One screening assay for identifying IL-1 receptor antagonists from a combinatorial library is
15   based on inhibiting IL-1 activity in a physiological setting. Such activities can be assessed in certain in vitro assays known in the art. Described below are representative in vitro assays useful in measuring IL-1 activity and blockade thereof. It is
20   appreciated that any of a number of known IL-1 activity assays can be used to assess inhibition of IL-1. Such assays may be substituted for the particular assays described below without deviating from the form of the present invention.

25   Alternatively, or in addition, IL-1 receptor antagonist compounds in a combinatorial library can be identified on the basis of their ability to displace IL-1 from its normal receptor sites on a cell surface. For example, the library compounds
30   may screened for their ability to displace reporter-labeled, e.g., radiolabeled, IL-1α or IL-2β from the surface of cells having a suitable IL-1 surface receptor, such as the EL-4 cells described in Example 7. The assay generally follows the
35   procedure disclosed in this example, for displacement of IL-1α or IL-1β.

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The identity of library compounds with observed inhibitory and/or binding displacement activity can be determined by conventional means, such as iterative synthesis methods in which sublibraries containing known residues in one subunit position only are identified as containing active compounds.

B.   Binding to IL-1R tI

Although IL-1 receptor antagonists that are useful in the present invention are not necessarily competitive antagonists of IL-1 at the IL-1R tI, compounds, such as IL-1ra, which bind competitively are preferred in at least one embodiment of the invention.   A determination that binding is competitive, as opposed to non-competitive, may be assessed using a number of pharmacological tools, including, for example, Schild plot regression analysis of dose-response curves generated from functional assays (Kenakin).

One assay for determining whether a compound binds competitively to an IL-1 receptor, is a competitive displacement binding assay (Mizel).   In this assay, as in the one detailed in Example 7, intact cells are incubated with ^{125}I-IL-1α, and binding to the cells is determined following separation from the cells of unbound ^{125}I-IL-1α.   In such an assay, competitive binding is evidenced by competitive displacement of bound ^{125}I-IL-1α from the cells when the cells are incubated with a test binding compound.

According   to   results   of   competitive displacement assays, such as that reported above, IL-1ra has approximately the same affinity as does IL-1 in binding to EL-4 cells, as well to 3T3 (fibroblasts) and CHO cells (McIntyre).   IL-1 receptor antagonists preferred for use in the

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present invention will have affinities for the IL-1 binding site, and more specifically, for IL-1R tI, which are substantially that of IL-1ra for the IL-1Rti. That is, preferred antagonists will exhibit

5   affinities that are higher than or only slightly lower than (K_i within about 2-3 log units) the affinity of IL-1ra for IL-1R tI, when compared in the same binding assay. In one cell binding assay that employs YTNC1 cells, IL-1ra had a K_i of about

10 0.25 nM. Accordingly, when measured in this system, it is anticipated that a receptor antagonist preferred for use in the present invention will have a K_i that is at least about 10^{-7} M.

It is also appreciated that activity of a

15 compound in a competitive displacement binding assay does not generally predict whether the test compound is an agonist or antagonist. Therefore, competitive displacement activity is not generally considered definitive of competitive antagonism. Compounds

20 shown to bind competitively to the receptor are tested in one or more functional assays, such as one or more of those described below, to determine whether binding to the receptor results in agonist or antagonist activity at the receptor.

25

C.   Functional Assays for IL-1 Receptor Antagonism

Functional or biological assays provide means of assessing whether a compound has agonist or antagonist activity at the IL-1R tI. Such

30 assessment is generally carried out by measuring activity of a known agonist compound in the assay, and comparing to this activity the activity of the agonist in the presence of test compound. In addition, the test compound is also evaluated in the

35 functional assay in the absence of the standard known agonist, to determine whether it possesses agonist activity as well. In general, preferred

20

antagonists will exhibit little or no agonist
activity, relative to antagonist activity.
Moreover, their antagonistic activity will be
substantially similar to that of IL-1ra, as
5   discussed below.

          1.   Mouse D10 cellular Proliferation Assay
          Example 8 details a method for assessing IL-1
     receptor antagonist activity in an assay in which
10   IL-1 stimulates proliferation of mouse T-helper
     cells.   Human IL-1α and human IL-1β stimulate
     proliferation of cells, measured by uptake of
     tritiated thymidine, in such an assay.   In this
     assay, 30 nM IL-1ra was effective to completely
15   inhibit the effects of IL-1α or IL-1β when the
     agonists were present at concentrations ranging from
     about 0.1 pM to approximately 50 nM.
          More generally, to assess relative antagonist
     potency in such an assay, IL-1α or IL-1β is added to
20   the assay at a half-maximal stimulatory
     concentration (EC50).   The concentration of IL-1ra
     or test compound is varied from about 10^{-12}M to 10^{-6}M,
     to determine a K_i value for the compound in the
     assay, according to standard procedures (Kenakin).
25   Compounds preferred for use in the invention will
     have a K_i that is substantially the same as a K_i
     determined for IL-1ra or icIL-1ra in the assay.
     That is, they will have a K_i that is less than about
     2-3 log units higher than the K_i determined for IL-
30   1ra or icIL-1ra in the assay.

          2.   Inhibition of serum IL-6 production
          A pharmacological effect of IL-1 is stimulation
     of IL-6 production in serum.   IL-1ra inhibits such
35   production, when given to mice in addition to IL-1
     (McIntyre).   Example 9 details a method by which IL-
     1 receptor antagonism is assessed in mice according

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to the ability of a compound to block such
stimulation. In this assay, as in the proliferation
assay, it is anticipated that preferred IL-1
antagonist compounds will behave substantially like
5   IL-1ra or icIL-1ra, as described for the
proliferation assay in sub-part 1 above.

IV.   IL-1 Receptor Antagonist-mediated Contraception
      A.   Involvement of IL-1 in Intrauterine
10   Function
      Certain paracrine cytokines are known to be
involved in interactions between the blastocyst and
endometrium. For example, secretion by the maternal
uterus of the leukemia inhibitory factor (LIF) has
15   been demonstrated to be required for successful
implantation of the fertilized ovum. Other
paracrine cytokines which have been implicated in
implantation include maternal colony stimulating
factor-1 (CSF-1), mutations of which have been shown
20   to compromise implantation. It is a discovery of
the present invention that disruption (prevention or
dislodgement) of embryonic implantation can be
effected *in vivo* by blockade in the maternal
endometrium of the endogenous cytokine IL-1 receptor
25   type I (IL-1R ti).

      The interleukin-1 (IL-1) system includes IL-1α,
IL-1β, IL-1 receptor antagonist (IL-1ra) and the IL-
1 receptor (IL-1 R). Two IL-1 receptor subtypes
have been identified. They are termed IL-1R type
30   I (IL-1R tI (Sims)) and IL-1R type II (IL-1R tI
(Horuk)). Both IL-1α and IL-1β bind to IL-1R tI
(Dower), and their effect on this receptor is
antagonized by IL-1ra.

      IL-1 and its receptor have both been localized
35   to endometrial cells in mice and humans. In
addition, IL-1α and IL-1β have been shown to be
secreted by the human embryo prior to implantation.
The IL-1 receptor (IL-1R tI) is also expressed in

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the human endometrium throughout the menstrual
cycle.   The agonist IL-1 has been used to induce
pre-term parturition in mice, and the antagonist IL-
1ra inhibits this abortifactant effect of IL-1
5   (Romero).   In contrast to the anti-abortive
effects of IL-1ra described above, as described in
the following sections, an important aspect of the
present invention is the discovery that antagonism
of IL-1r tI by receptor antagonists is effective to
10   prevent implantation of a fertilized ovum, when IL-
1ra is present in the uterus during the implantation
event, as well as to dislodge such implantation
after it has occurred. More generally, according to
these findings, it can be stated that IL-1 receptor
15   antagonism is effective to provide contraception
during the peri-implantation period. This period is
a time during which the fate of the embryo can be
manipulated by hormonal or biochemical means related
to the implantation process. In the context of the
20   present invention, this time period refers to the
time period during which administration of IL-1ra
to the subject can disrupt, that is, prevent or
reverse implantation, as described in Section V
below.
25   Additionally, studies carried out in support of
the present invention indicate that IL-1 receptor
antagonism is effective to prevent ovulation in
animals stimulated to ovulate, as described in sub-
section D, below.
30

    B.   Embryonic Regulation of Expression of IL-1
          Receptor in Uterine Endothelial Cells

        Experiments carried out in support of the
present invention show that fertilized ova secrete
35   substances into their surroundings which, when
brought into contact with uterine endometrial cells,
stimulate the cells to express increased levels of
the IL-1 receptor type I (IL-1R tI).

23

Figures 1 and 2 show results of experiments in which human uterine endometrial stromal cells (ESC) and endometrial epithelial cells (EEC), isolated from ovulatory women in the luteal phase, were
5   cultured and grown in basal conditions in steroid-free media, as detailed in Example 1. The resulting monolayers were maintained for 6 days under the growth conditions prescribed by an established model for *in vitro* decidualization
10   (Irwin), as detailed in Example 1. At that time, one of the following embryonic products was added to the endometrial culture medium: human recombinant IL-1b (rIL-1b), Human Chorionic Gonadotropin (HCG), Platelet activation factor (PAF), or conditioned
15   media from human blastocyst (HBCM; prepared as described in Example 1). Cells were subjected to RNA analysis by Northern blot with human IL-1R tI cRNA probe, according to established procedures, as described in Example 1. For quantitation of
20   results, Northern blot data were normalized to a standard 28S rRNA cDNA probe standard, according to established methods (Simon).

As shown in Figures 1 and 2, IL-1R tI mRNA and immunoreactive IL-1R tI were found in both human ESC
25   and EEC. In human ESC, cultured under basal conditions in the presence of progesterone (P) and epidermal growth factor (EGF) only IL-1R tI mRNA expression was upregulated 2.6-fold after 8 days in culture. Addition of embryonic products to the
30   cells for 2 days (from day 6 to 8) produced a further up-regulation of IL-1R tI as follows: conditioned media from human blastocyst (7.5-fold increase), PAF (5.7-fold increase), IL-1b (4.2-fold increase), hCG (3.5-fold increase) (Figures 1A and
35   1B).

In EEC, IL-1R tI mRNA constitutively expressed at higher at levels than those observed in ESCa.

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However, in these cells, IL-1R tI mRNA was not significantly regulated by any of the embryonic products observed to increase receptor expression in ESC (Figures 2A and 2B).

5

C.   Preventing Embryonic Implantation
      1.   Murine   Model   of   Embryonic
            Implantation.

A mouse model was used to study implantation.

10 The validity of the mouse model to the study of implantation in humans was assessed by determining (a) the presence in the mouse uterus endometrium of immunoreactive IL-1R tI following pregnant mare's serum gonadotropin (PMSG) and human chorionic

15 gonadotropin (hCG) administration, and (b) the presence of IL-1R tI in the epithelial region surrounding the blastocyst during the peri-implantation period, specifically at day 4 of pregnancy, since these conditions have been observed

20 in human uterine tissues. Other indicators of the validity of the murine model to human conditions are known in the art (Clark). Specifically, IL-1α and IL-1β mRNAs and Il-1 bioactivity in the peri-implantation uterus are known to increase from day

25 3 and peak between days 4 and 5, post-fertilization. Blastocyst implantation generally occurs late on post-fertilization day 4 in mice, but may be delayed until about day 8, due to the phenomenon of "delayed implantation" which is apparently inherent to mice.

30   Figure 3 shows the results of experiments in which immunoreactivity of IL-1R tI and IL-1β were measured in the mouse maternal endometrium and in the mouse placenta, respectively, following fertilization, as detailed in Example 2. As shown,

35 mouse IL-1R tI is localized in the apical region of the lumenal epithelium and in scattered areas of the stroma (Figures 3A and 3B). Increased IL-1R tI

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immunoreactivity was observed in the epithelial
tissue surrounding the blastocyst, as indicated by
the arrow in Figure 3D.   As shown in Figure 3E, at
day 9 of pregnancy, immunoreactive IL-1β was present
in the mouse placenta (p) with increased staining at
the interface with the maternal endometrium (m).
These results serve as biochemical validation of the
mouse model for human implantation, with respect to
the IL-1 cytokine system, as described above.

Although   gestational   length   differs
considerably between mouse and human, since certain
common developmental stages can be identified,
comparable timepoints can be established between the
two species. Extrapolation of critical gestational
timepoints from mice to humans is known in the art
to be within the skill of the practitioner; however,
when appropriate, such extrapolations are also
provided herein.

2.   Time Course of Embryonic Implantation

For the purposes of the present discussion, the
female reproductive system comprises the ovaries,
Fallopian tubes or oviducts, uterus and vagina. Ova
mature in the ovarian follicles from oocytes
contained within. Mouse and humans ovulate (release
mature ova from the follicles) in response to
approximately the same hormonal stimuli, which
include a periodic spike of luteinizing hormone (LH)
produced in the pituitary and measurable in the
serum. Ovulation in mice occurs spontaneously about
every 4 days; in humans, ovulation occurs about
every 28 days.

Following ovulation, the ovum is taken into an
oviduct, where it is thought to remain fertile for
10-24 hours in humans and for about 12 hours in
mice. Fertilization, if it occurs, generally occurs

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in the ampulla of the fallopian tube in humans, also known as the oviduct in mice.

In the mouse, following the release of sperm into the female reproductive tract, the sperm undergo capacitation, a process that renders them competent for fertilization. Capacitation usually occurs about 1 hour following ejaculation. Mouse sperm generally remain viable in the female reproductive tract for about 6 hours after ejaculation. Human sperm generally remain viable for at least about 48 hours after ejaculation. Generally, according to these timepoints indicate it is thought that for fertilization to occur in humans, coitus should occur no more than about 48 hours before or 24 hours after ovulation.

Following fertilization, the maturing embryo migrates to the uterus. During this migration, the embryo undergoes maturation through cell division. By the time the embryo reaches the uterus, generally about 2.5-3 days post-fertilization in mice and about 4 days post-fertilization in humans, it has progressed through the morula stage to the blastocyst stage. At the uterus, the embryo, generally in morula or blastocyst stage, attaches to the uterine wall in a process known as implantation. In humans, implantation occurs between days about 4 to 7, and particularly 5.5 to 6 after ovulation. In mice, implantation usually takes place between day 4.5 and day 5 following ovulation, though it can be delayed until about day 8, due to the delayed implantation phenomenon discussed above.

3.   Inhibition of Embryonic Implantation
      by Blockade of IL-1 Receptors

It is one discovery of the present invention that blockade of the IL-1 receptor can prevent embryonic implantation, thereby interfering with

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pregnancy. In experiments carried out in support of
the invention, Il-1ra was administered by
intraperitoneal injection to female mice previously
mated with males of the same strain and age, and
5   outcome was assessed by measuring the number of
successful implantations, as detailed in Example 3.
Briefly, 12-week-old B6C3F-1 female mice were super-
stimulated to ovulate by administration PMSG/hCG.
Stimulated females were mated with males of the same
10   strain and age. Coitus was assessed by detection of
vaginal sperm plug 24 hours after hCG injection. At
day 3 of pregnancy, mice were divided in 3 groups:
control non-infected, control buffer injected, and
mice injected with IL-1ra.
15   Results of the mouse implantation studies are
shown in Table 1. As indicated, treatment of female
mice with IL-1ra resulted in a significant decrease
in the pregnancy rate ($p=.000115$ compared to control
buffer injected and non-injected animals (Fisher
20   exact test). These results indicate that post-
fertilization administration of IL-1ra is effective
to interfere with pregnancy.

Table 1

25   Effect of rh IL-1ra Administration on the
Reproductive Outcome of Pregnant Mice

Control Group Non-Injected (n = 17)	Control Group Buffer Injected (n = 19)	IL-1ra Injected Group (n = 15)
Pregnant = 10 (58.8%)	Pregnant = 14 (73.3%)	Pregnant = 1 (6.7%)
Non Pregnant = 7 (41.2%)	Non Pregnant = 5 (26.3%)	Non Pregnant = 14 (93.3%)

35

    In further support of the invention, a
morphologic longitudinal study was carried out to
monitor embryonic implantation in the mouse from
pregnancy day 4 to day 9 in control, untreated

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animals compared with hr IL-lra treated animals, as
described above.   Fig. 4 shows microscopic sections
of mice uteri made at various days post-
fertilization.   With reference to Figure 4A,
5   showing a longitudinal section of the uterus at day
4. Here, the free blastocyst is located within the
lumen of the uterus. With reference to Figure 4C
and Figure 4F, showing sections taken at days 7 and
9 of normal pregnancy, respectively, the mouse
10   embryo is observed to be completely implanted in the
stroma.

        In   the   IL-lra   treated   animals,   the   free
blastocyst was also found within the uterus by post-
fertilization day 4, shown in Figure 4B. By day 7
15   the embryo in the IL-lra treated animal had neither
attached nor implanted (Fig 4D). At day 9, no
stromally implanted embryos were observed in the IL-
lra treated animal; however degenerated trophoblast
cells were found in the uterine lumen (Figure 4F).

20   The studies described above indicate that
embryonic IL-1 binding to maternal IL-1R tI is
required for successful implantation of the embryo
in the uterus, since a specific antagonist of this
binding prevents the implantation process. These
25   studies also provide basis for the contention that
plentiful distribution of IL-1R tI throughout the
lumenal epithelium is required for initiation of
receptor-ligand interaction initiated wherever the
embryo attaches. Once the embryo has traversed the
30   epithelium and begins stromal invasion, its own
secretion of IL-1, and perhaps other paracrine
factors, induces IL-1R tI in the surrounding stroma,
allowing implantation to proceed. However, as shown
herein, blockage of the IL-1R tI by an antagonist is
35   sufficient to prevent implantation.

        Evidence that the effects observed in the mouse
model were specific effects, rather than non-

specific toxic effects on the embryo was provided by
experiments in which early (2-cell) mouse embryos
were flushed from the oviducts of the same group of
mice used for *in vivo* experiments. The embryos were

5   incubated with increasing concentrations of rh IL-
1ra for three days, as detailed in Example 4A.
Assessment of viability was made, based on the
number of embryos that reached blastocyst stage
after 72 hours in culture. As indicated by the

10   results presented in Example 4, no significant
differences in survival were noted between embryos
incubated in the presence of concentrations of IL-
1ra ranging from 0 to 200 μg/ml.

Recombinant human IL-1ra was further tested for

15   possible effects on later developmental stages of
the mouse embryo, including blastocyst hatching,
trophoblast outgrowth and migration, as detailed in
Example 4B. Blastocysts were cultured 5 days on
fibronectin-coated plates in the presence of absence

20   of Il-1ra, as described in Example 4B. Hatching,
outgrowth and migration were found to be similar in
the presence or absence of rh IL-1ra. These
experiments demonstrate that rh IL-1ra is not toxic
to the embryo itself and provide further evidence

25   that the contraceptive effects of IL-1ra are
specific to the implantation process.

D.   Dislodging Implanted Embryos

It is also an observation of the present

30   invention that interference with implantation can
occur after the initial implantation event. To be
effective, an IL-1R tI antagonist compound will
preferably be administered during the post-
implantation period, which extends from the

35   implantation period to at least several days
following the initial implantation event, as
indicated in Figure 5.

30

E.   Prevention of Ovulation

A further observation of the present invention is that IL-1 receptor antagonism is effective to inhibit or prevent ovulation in animals stimulated

5   to ovulate. In studies carried out in support of the present invention, and described in Example 10, female rats were primed with gonadotropin. Prior to stimulation with hCG, the animals IL-1ra was administered to the animals, and ovulation was

10 significantly inhibited.

V.   Contraceptive Compositions and Methods of Treatment

A.   Methods of Contraception

15   1.   Prevention of Ovulation and Implantation

The invention includes in one aspect a method of contraception by prevention of implantation of the fertilized ovum or embryo. This aspect of the invention may also include prevention of ovulation.

20 According to this method, an IL-1 receptor antagonist is delivered to the uterus during the time period between coitus and the implantation period; that is, during a period 1-2 days before ovulation or 3-7 days after ovulation. The

25 significance of these time periods will become more apparent with reference to the temporal events depicted in Figure 5, as described below.

IL-1 receptor antagonists for use in preventing ovulation and embryonic implantation are selected as

30 described in Sections II and III, above. That is, suitable IL-1 receptor antagonist compounds are selected for their ability to (i) bind to the IL-1 tI receptor, and/or (ii) antagonize the effects of IL-1α or IL-1β at the receptor. Such compounds will

35 exhibit a pharmacological profile that is substantially similar to that of IL-1ra or icIL-1ra, and will have very little or no IL-1 agonist

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activity.   IL-1ra and derivatives thereof may be
used in the method of treatment, in accordance with
the working examples described in Section IV, above.

5   Figure 5 shows a temporal depiction of the
human menstrual cycle, in which day 1 indicates
initiation of menstrual flow. Generally, ovulation
occurs at about day 14 of the cycle, as depicted;
however, this may vary considerably in and among
individual females.   In determining the pre-
10   implantation period, it is necessary to refer to the
day of ovulation, such a depiction must be taken as
a convenient guideline, rather than a standard. For
the purposes of determining the peri-implantation
period in an individual, the reference point is
15   preferably the day of ovulation.   Generally,
ovulation occurs just subsequent to a spike of LH
and FSH levels in serum. These and other hormonal
or physiological measures of ovulation may be
measured according to standard methods known in the
20   art. Alternatively, the day of coitus may be used
as a reference point, in which case the pre-
implantation period will extend to the outer limits
shown in Figure 5, as described below.   A   s
described above, in humans, sperm are thought to
25   remain viable in the female uterus and fallopian
tubes for at least 2 and possibly as long as 5 days
following coitus. The ovum is viable for about 24
hours following ovulation.   Using the 2 day
viability period for sperm, a fertilization period
30   of 3 days (0-3 days post-coitus (p.c.); days 12-15
in the idealized menstrual cycle) is indicated in
Figure 5.

Since in humans, implantation generally takes
place between about 4 and 7 days after ovulation, it
35   can be appreciated, with reference to Figure 5, that
this time will be from about day 3 to about day 11
p.c., assuming that coitus may have occurred at any

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time between day 12 and day 15 of the idealized
cycle.   This implantation period is indicated as
days 18-22 in the menstrual cycle.   These events,
coupled with the animal studies reported in Section
5   IV, define a pre-implantation period that ranges
from about day 12 to about day 18 of an idealized
menstrual cycle.   Preferably and practically, in
accordance with the present invention, it will be
appreciated that prevention of implantation will be
10   effected by administration of an IL-1 receptor
antagonist between about day 3 post-ovulation and
about day 7 post-ovulation.   When the day of
ovulation is uncertain, it will be assumed that it
spans the three day fertilization period shown in
15   Figure 5.   In such a case, compound will preferably
be administered between about day 1 and about day 9
p.c.   More preferably, the compound will be
administered between about day 3 and day 7 p.c.

In accordance with the invention, it is
20   appreciated that an IL-1 receptor antagonist will
also be effective to inhibit ovulation, in the case
where ovulation has not occurred prior to
intercourse, such as during the period 1-2 days
before ovulation (days 12-14 in the idealized cycle
25   illustrated in Figure 5).   In such a case, if the
time of ovulation is uncertain, the compound will be
effective if administered to the subject between
about day 0 and day 9 post-coitus.

30   2. <u>Dislodging the Implanted Embryo</u>
With continued reference to Figure 5, it will
be appreciated that prevention of pregnancy can also
be achieved, when IL-1 receptor antagonism
interferes with implantation after the initial
35   implantation event. According to the cycle shown in
the figure, where implantation occurs between day 18
to day 21 of the cycle, post-implantation

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interference, or "dislodgment" will occur as early as day 18 and as late as day 28 in the cycle. Preferably, such dislodgement will be effected between about day 4 and day 10 following ovulation.

B. Pharmaceutical Compositions

The dosage of IL-1 receptor antagonist required to disrupt implantation will depend on the specific compound and the route of administration, as described below. Generally, an effective dosage will be a dosage that provides in the uterus a concentration that is equivalent to a concentration of IL-1ra of about 1-50 μg/ml. In humans, serum concentrations of about 30 μg/ml have been achieved by administering an intravenous dose of 10 mg/kg IL-1ra (Granowitz).

Although any mode of administration may be used, so long as it provides sufficient concentration of compound to the uterus, it can be appreciated that certain modes of administration will be preferred in the treatment method. In the case of IL-1ra, intravenous administration is possible; alternatively, for convenient self-administration by the patient, other modes of administration, such as nasal insufflation or intravaginal insertion may be preferable. The relative pharmacokinetics of such modes of administration are known in the art.

In one preferred embodiment, the IL-1 receptor antagonist will be formulated in an intravaginal suppository insert having slow release properties, such as are afforded by the formulation described in U.S. Patent No. 5,116,619, incorporated herein by reference. This formulation, which includes, in addition to the active ingredient, about 65-85% lactose, 2-4% starch paste, 2-4% corn starch and 0.4-0.6% magnesium stearate, allows for prolonged

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release of active ingredient over 48-72 hours. It can be appreciated that such a suppository formulation can be dispensed for alternate night self-administration, during the peri-implantation period determined according to the practitioner's understanding of the particular patient's ovulatory cycle and the principles taught herein.

Another preferred administration mode includes the use of a cervical cap to deliver IL-1ra to the uterus through the cervix. Cervical caps, such as the cervical cap described in U.S. Patent No. 4,961,436 incorporated herein by reference, are known in the art, and may be used to deliver over the peri-implantation period, a medicament containing an IL-1 receptor antagonist effective to produce in the uterus a concentration of the antagonist equivalent to about 1-50 μg/ml IL-1ra.

In accordance with current techniques in transdermal iontophoresis of peptides, it is also appreciated that IL-1 receptor antagonist peptides may be administered by transdermal delivery, as through "patch" application. Example 4C. describes a system suitable for transdermal delivery of a peptide formulation suitable for use in the methods of the present invention.

The following examples illustrate, but in no way are intended to limit the present invention.

Materials

Interleukin 1α (IL-1α) and Interleukin 1β (IL-1β) were obtained from Genzyme (Norwalk, CT) and are also available from Gibco (Grand Island New York).

All other reagents may be obtained from the suppliers listed in the text or are standard in the field.

35

Example 1
Localization of IL-1 and IL-1R tI
in Uterine and Embryonic Tissues

A.   Culture of Endometrial Cells

5   Human endometrial samples from ovulatory women
undergoing hysterectomy for non malignant
indications were obtained in the secretory phase.
Endometrial stromal cells (ESC) and epithelial cells
(EEC) were isolated according to standard
10   procedures, cultured, and grown in basal conditions
in steroid-free media supplemented with 10% serum
until confluent monolayers were formed. Endometrial
tissues samples were minced into small pieces of
less than 1 mm, and subjected to mild collagenase
15   digestion as follows: Tissue pieces were incubated
with shaking for 2 hours at 37°C, in DMEM (Gibco,
Grand Island, NY) containing 0.2% collagenase Type
I and 0.005% deoxyribonuclease Type I (Worthington,
Freehold, NJ). ESC and EEC were isolated from the
20   mixture by centrifugation, then cultured, and grown
to confluence in 75% Dulbecco's Modified Eagle's
Medium (Gibco, Grand Island, NY) and 25% MCDB-105
(Sigma, St. Louis, MO), containing antibiotics,
supplemented with 10% serum (Charcoal-Dextran
25   treated FBS, Hyclone, Logan, Utah) and 5 μg/ml
insulin (Sigma, St. Louis, MO). ESC and EEC were
grown in steroid free medium until they were
confluent monolayers. The homogeneity of cultures
was determined by morphological characteristics and
30   verified by immunocytochemical localization of
cytokeratin, vimentin, and CD68 antigen as markers
for epithelial cells, stromal cells, and human
macrophages, respectively. As defined by these
criteria, ESC monolayers contained less than 2% of
35   EEC and less than 0.1% of macrophages. EEC cultures
contained less than 3% of ESC and less than 0.1% of
macrophages.   After cells were confluent (1-2
weeks), the media was supplemented with 5 μg/ml

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ascorbic acid, 10 μg/ml transferrin and hormones, as indicated below.

Monolayers were cultured for 6 days with progesterone (P) (10 nM) and epidermal growth factor
5   (EGF) (3.3 nM), according to an established model for *in vitro* decidualization (Irwin). On day 6, human recombinant IL-1b (hrIL-1b) (10 IU/ml), human chorionic gonadotropin (HCG) (10 IU/ml), platelet-activating factor (PAF) (200 nM), or conditioned
10 media from human blastocyst (HBCM; 1ml) were added to the media and cells were further cultured for 2 days and compared with control cells cultured only with P and EGF.

HBCM is conditioned media (500-1000 ml) from
15 human embryos cultured *in vitro* until blastocyst stage. To prepare HBCM, embryos were cultured in Human Tubal Fluid medium (HTF Medium, Irvine Scientific, Santa Ana, CA) containing penicillin (0.5 mg/ml), streptomycin (0.5 mg/ml) and 1% human
20 serum albumin (Fraction V, Irvine Scientific) for approximately 4 to 5 days. The medium covering these cells was then collected, frozen, and stored at -70°C degrees until use. Growth medium was renewed every 2 days, and cells were removed every
25 2 days for RNA analysis.

    B.   Regulation   of   IL-1ra   Expression   by
          Embryonic Factors

RNA   from cultured endometrial cells was
30 analyzed by Northern analysis with human IL-1R tI cRNA probe. A 28S rRNA cDNA probe was used to normalize the data as according to standard methods (13, 14). Data were expressed as arbitrary units:

$$\frac{\text{IL-1R tI mRNA}}{\text{28S rRNA}}$$

35

Total RNA was extracted from ESC cells according to methods known in the art (Chomczynski),

then 10 μg samples were fractionated by electrophoresis according to standard methods (Ausubel) and transferred to a nitrocellulose membrane. The filters were hybridized with a cRNA probe for human IL-1R tI (Simms). Equal loading of RNA on the filters was confirmed by densitometric analysis of 28S rRNA cDNA hybridization signals. IL-1R tI mRNA levels were normalized to 28S rRNA levels and expressed as an arbitrary unit, the IL-1R tI mRNA/28S rRNA level at day 0 of treatment. Densitometric analysis of Northern blots of IL-1 R tI mRNA from different experiments were combined and expressed as the mean ± standard deviation (sd). Protein studies were performed by immunocytochemistry using both indirect immunofluorescence and avidin-biotin methods (Simon).

ESC were cultured until confluence in steroid-free conditions as detailed above, RNA extracted, transferred and hybridized with IL-1R tI cRNA probe. Figures 1A and 1B shows Northern blots and a bar graph of quantitation of IL-1R tI mRNA in cultured human endometrial stromal cells (ESC). In the bar graphs combine values obtained from several experiments as indicated by "n". Values are expressed as mean ± standard deviation (sd). The positions of 18S and 28S ribosomal RNA are indicated to the left of the Northern blots.

With reference to Figure 1A, lane 1 IL-1R tI mRNA expression at day 0, prior to any further treatment of cells. This lane serves as control. IL-1R tI mRNA levels of ESC treated with progesterone and epidermal growth factor (P+EGF) for 6 and 8 days are shown in lanes 2 and 3, respectively. Lanes 4-7 show blots from ESC treated with P+EGF for 6 days, then, treated for 2 additional days (until day 8) with media from human

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blastocyst (1 ml total, Lane 4), 200 nM PAF (Lane
5), IL-1b 10 IU/ml (Lane 6), or hCG 10 IU/ml (Lane
7).

5   Figures 2A and 2B show the same representations
described above for Figures 1A and 1B for
endometrial epithelial cells (EEC). EEC cultured
until confluence in steroid free-conditions were
treated as described for ESC, above. Treatments and
lanes correspond to those described above for ESC
10   treatment.

Example 2
Immunodetection of IL-1R tI and IL-1β

A female mouse was given 5 IU pregnant mare
15   serum gonadotropin (PMSG) by I.P. injection.
Twenty-four hours later, the uterus was removed and
stained with rat monoclonal anti-mouse IL-1r TI
antibody, according to standard immunohistochemical
methods (Simon, *et al.*) (14). Experiments were
20   repeated with at least three different samples in
each case, unless otherwise indicated herein.

Figure 3 shows the results of studies using
indirect immunofluorescence to localize of
immunoreactive endometrial mouse IL-1R tI (A-D) and
25   immunoreactive IL-1β (E) in the mouse embryo.
Figure 3 (A-E) shows results (A) Mouse uterus
obtained 24 h. after pregnant mare serum
gonadotropin (PMSG) injection (5 IU); mouse IL-1R tI
is localized in the apical region of the lumenal
30   epithelium and in scattered areas of the stroma;
(original magnification x400). (B) Mouse uterus
obtained 24 h. after hCG injection (5 IU); Stronger
IL-1R tI staining throughout the epithelium and in
the stroma is observed; (x400). (C) Negative control
35   by deletion of the primary antibody; (x400). (D)
Mouse uterus obtained at day 4 of pregnancy (single
experiment). Increased staining was observed for

IL-1R tI in the epithelium surrounding the
blastocyst (arrow); (x400).

Figure 3E shows immunolocalization of mouse
embryonic IL-1b, at day 9 of pregnancy with twin
5   embryos (indicated at "e" in the figure)
immunoreactive IL-1b is present in the mouse
placenta (indicated at "p" in the figure) with
increased staining at the interface with the
maternal endometrium (indicated at "m" in the
10   figure); (x400). Monoclonal antibodies against mouse
IL-1b and the extracellular domain of the mouse
IL-1R tI were acquired from Genzyme (Cambridge, MA).

Example 3
15   ## Inhibition of Embryonic Implantation *in vivo*
A.   Periodic Injection of Il-1ra
Female 12-week-old B6C3F-1 female mice
(approximately 20 g) were stimulated with 5 I.U. of
Pregnant Mare's Serum Gonadotropin (PMSG; Sigma, St.
20   Louis, MO) i.p., followed 24 hours later by 5 I.U.
human chorionic gonadotropin (hCG) i.p.
administration. The mice were then mated
immediately with males of the same strain and age.
Females with vaginal plugs (designated pregnancy day
25   1) were randomly allocated to three groups: A,
Control non-injected; B, Buffer injected animals and
C, animals injected i.p. with 20 μg of recombinant
human IL-1ra (rh IL-1ra) every 12 hours beginning on
pregnancy day 3 and ending on day 9.
30   Intraperitoneal injections were carefully directed
away from the pregnant uterine horns. Injections
were continued until day 9 to avoid the possibility
of delayed implantation, and animals were sacrificed
12 hours after the last injection. Animals appeared
35   healthy throughout the experiment. Implantation
sites in both uterine horns in IL-1ra injected
animals were counted and compared with control

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animals receiving buffer or no injection. Two
different preparations of IL-1ra (26) and two
different buffers were used in four separate
experiments. Data were analyzed by Fisher Exact
5   Test. Results of these studies are shown in Table
1, in Section IV.C.3, above.

        B.   Morphological Assessment of Effects of IL-
              1ra on Embryonic Implantation

10   PMSG/hCG stimulated 12-week-old B6C3F-1
females were mated with males of the same strain and
age. Females with vaginal plugs (designated
pregnancy day 1) were randomly allocated to two
groups: I, Buffer injected animals, and II, animals
15 injected i.p. with 20 μg of recombinant human IL-1ra
(rh IL-1ra) (26) every 12 hours beginning on
pregnancy day 3 until day 9. Starting on day 3, one
mouse from each group was sacrificed each day.
Uteri were removed and immediately fixed in 10%
20 formalin in phosphate buffered saline. Fixed tissue
were embedded in paraffin, sectioned and mounted on
glass slides. Serial sections (4 μm) from each
sample were then stained with hematoxylin-eosin
(H&E) and viewed with an Olympus 35 mm camera and an
25 Olympus BH2 microscope.
        Results are shown in Figure 4 (A-F).
Micrographs from untreated animals are shown in
Figures 4A, 4C, and 4E. Human recombinant IL-1ra
treated animals are shown in figures 4B, 4D, and 4F.
30 Figure 4A shows day 4 normal pregnancy; longitudinal
section of a free blastocyst surrounded by enlarged
uterine lumenal epithelial cells [Hematoxylin/Eosin
(H & E), original magnification x400]. Figure 4B
shows Day 4 pregnant animals injected with IL-1ra;
35 Cross-section of a free blastocyst. In this
micrograph, the epithelium surrounding the
blastocyst is not enlarged as observed with normal
pregnancy (H & E x400). Figure 4C shows the uterus

of day 7 normal pregnant mouse; longitudinal section through embryo and decidual mass demonstrating complete stromal implantation (H & E x400). Figure 4D shows the uterus of day 7 of pregnant mouse,

5   IL-1ra injected. Here the blastocysts are still free. No attachment or invasion is observed (H & E x400). Figure 4E shows the uterus of day 9 normal pregnant mouse; longitudinal section through embryo and decidual mass (H & E x200). Figure 4F shows the

10 uterus of day 9 of pregnancy, IL-1ra injected mouse; stromal decidual reaction (arrows) with intact glandular epithelium and degenerated trophoblastic cells in the uterine lumen (arrowhead) can be observed, but implantation did not occur in this

15 animal (H & E x800). This is a section from the single IL-1ra injected mouse described in Table 1 that was macroscopically pregnant.

C.   Transdermal delivery of IL-1 receptor

20   antagonist peptides

Female rodent subjects, preferably hairless guinea pigs or mice (subjected to a depilatory agent) are mated, and examined for evidence of pregnancy as described in Part A, above. Animals

25 are then fitted with a transdermal patch system, according to methods described by Kumar, et al. (J. Controlled Release 18: 213-220, 1992), incorporated herein by reference. Briefly, the patch consists of a die cut reservoir ring of closed cell polyethylene

30 foam having contact adhesive on one side. The ring is fitted one side with a Millipore GVWP hydrophilic filter. The ring and the filter join to form an anode reservoir, into which is placed a gel containing IL-1 receptor antagonist. The gel

35 consists of Polyjel HV (polyglyceryl citrate + hydroxypropyl methyl cellulose; Guardian Chemical, Hauppauge, NY) mixed with acetate buffer (0.05 M, pH

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5.8) into which is mixed the peptide at about 0.1%
(wt/wt). The reservoir is closed with a transparent
polyethylene film having contact adhesive on one
side, into which is embedded a stainless steel snap
5   button electrode.   Alternatively, for use with
acidic molecules, a cathode patch, consisting of a
karaya gumpad (Iomed, Inc., Salt Lake City, UT) is
used.

The patch is connected to a pulsed voltage
10   output power source with a current limiting feature.
Animals are treated using a current limit of ·0.17
mA/cm^2 at 50 KHz and 50% duty cycle or 0.85 mA for a
total area of 5 cm^2, for 5 hours. Blood is sampled
for presence of the peptide, to determine the
15   delivered dose.   Control animals are given the
carrier gel, without IL-1 receptor antagonist
compound.

After an appropriate gestational period, as
described in part A above, animals are then tested
20   for presence implanted embryos, as described in
Example 3, part A, above.

<u>Example 4</u>
25   <u>Effect of Il-1ra on Embryonic Development</u>
A.   <u>Culture of Early Embryos with IL-1ra</u>
2-cell mouse embryos were flushed from the
oviducts on day 1 of pregnancy from animals
impregnated as described in Example 3.   Embryos
30   (n=276) were rinsed and placed in 4-well Nuncon
plates containing 500 μl of human tubal fluid medium
(HTF Medium, Irvine Scientific Santa Ana, CA) with
0μg/ml (n=91), 1μg/ml (n=36), 50 μg/ml (n=36), 100
μg/ml (n=52), and 200 μg/ml (n= 61) of rh IL-1ra.
35   After 72 h in culture the percentage of embryos
reaching the blastocyst stage was 85.7%, 91.6%,
94.4%, 96% and 85.2% respectively .

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B.   **Effects   of   IL-1ra   on   Blastocyst Development**

Effects of rh IL-1ra on blastocyst hatching, trophoblast outgrowth and migration, were determined by further culture of the blastocysts for 5 days on fibronectin-coated plates (Upstate Biotechnology, Inc., Lake Placid, NY, 27). Ten blastocysts obtained from control wells (0 μg/ml rh IL-1ra) and 10 from wells grown in the presence of 200 μg/ml of rh IL-1ra were placed in plates containing 200 μg/ml of rh IL-1ra.   Ten blastocysts from control and IL-ra containing cultures were placed in control plates lacking IL-1ra. In both groups, hatching, outgrowth and migration were documented to be similar in the presence or absence of rh IL-1ra.

Example 5
Recombinant Expression of IL-1ra

Plasmids containing the human IL-1 receptor antagonist (hIL-1ra) gene were obtained Immunex Corporation, Seattle, WA. The gene sequence of the insert is shown as SEQ ID NO: 1 in Figure 6 herein. Alternatively, the gene can be synthesized using an oligonucleotide synthesizer, or obtained by the isolation/synthetic   method   described   in   the reference by Carter, et al., incorporated herein by reference, and verified, using the sequence shown as SEQ ID NO: 1.   Alternatively, primers based on this sequence can be used for preparation of the gene sequence,   according   to   standard   procedures (Ausubel).

For use in experiments carried out in support of the present invention, recombinant *E. coli* human IL-1ra was expressed as a fusion protein using the pMAL vector (New England Biolabs). The expressed fusion protein was detected and purified from the periplasm of *E. coli* by Amylose affinity chromatography (New England Biolabs). The amylose

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eluate pool was dialyzed into 50 mM Tris-HCL, 100 mM
NaCl pH 7.5 and cleaved with Factor Xa (Enzyme
Research Labs) at a concentration of 10 ng to 50 µg
of fusion protein. SDS-PAGE was run to detect Factor
5   Xa cleavage of the fusion protein. Homogeneity of
hIL-1ra was achieved with further purification onto
Q-Sepharose pH 8.5 and Sephacryl S-300 gel
filtration chromatographies (Pharmacia). The
purified protein has a molecular mass (Mr) of 17,
10 000 dalton. Human Il-1ra activity was measured by a
mouse D10 cell proliferation assay, protein
quantitation by gel laser densitometer (Molecular
Dynamics) and endotoxin level was measured by the
Limulus assay (Whittaker Bioproducts).
15

Example 6
Purification of IL-1ra

IL-1ra is purified from cellular sources as
described by Carter, et al. as follows: U937 cell
20 cultures are obtained from the American Type Culture
Collection, Rockville, MD. Cells are cultured at
37°C for 48 h at 5×10^5 cells ml^{-1} in RPM1 1640
medium (GIBCO), 7% fetal bovine serum (FBS, GIBCO),
2mM L-glutamine, 100 U ml^{-1} penicillin, 100 µg ml^{-1}
25 streptomycin, 20mM HEPES buffer, and 100 nM phorbol
myristate acetate (PMA) (Sigma, St. Louis, MO).
After differentiation, the medium is removed and the
cells, now adherent, are gently washed once with a
small volume of Dulbecco's PBS. Cells are then
30 cultured for an additional 48 h in RPM1 1640
containing 1% low-endotoxin FBS (HyClone, Logan,
UT), L-glutamine, penicillin, streptomycin, and
rhGM-CSF (75 U ml^{-1}; Amgen, Torrance, CA). Cell-free
supernatants are collected, pooled and frozen at -
35 20°C. After thawing, phenylmethylsulphonyl fluoride
is added to 0.2 mM final concentration. The
solutions are concentrated by ultrafiltration with

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YM-5 and YM-10 filters (Amicon). Protein
measurements are made using Bradford assay (Bio-Rad
Kit) using BSA as the standard protein.

The preparation is then subjected to Superose
12 FPLC-1 chromatography in 17 repetitive cycles.
Two-ml aliquots of Fraction 2 concentrate are
injected onto a Superose 12 prep grade FPLC column
(1.6 x 50 cm) equilibrated with 0.1 M potassium
phosphate, 1 M NaCl, pH6.0. Fractions of 1 ml are
collected at a flow rate of 1 ml min^{-1}. Fractions
are collected and individually concentrated by
Centricon-10 units (Amicon). After analysis by SDS-
PAGE and bioassay for IL-1ra activity, appropriate
fraction concentrates are pooled and further
concentrated to 1 ml by Speed Vac centrifugation
(Savant).

Pooled samples are then subjected to TSK Bio-
Sil 125 HPLC-1, as follows: in 20 repetitive cycles,
50 μl of fraction 3 are injected onto a TSK-Bio-Sil-
125 HPLC column (7.5 x 600 mm), using the same
buffer system as described above. Fractions of 200
μl are collected. Appropriate fractions are pooled
and concentrated by Centricon-10 filter units and
finally by Speed Vac centrifugation as described
above.

Pooled samples are further purified, using TSK
Bio-Sil 125 HPL-2, as follows: in seven repetitive
cycles, 20 μl of fraction 4 are injected onto the
same column as described above. Active fractions
are pooled to give a final volume of 8.5 ml, and are
subjected to C4 reverse phase HPLC, as follows:
appropriate fractions from the TSK Bio-Sil 125 run
(fraction 5) are injected directly onto a C4 reverse
phase column (4.6 x 150 mm). Proteins which remain
bound to the matrix following an extensive wash with
0.1% trifluoroacetic acid (TFA) are desorbed using
a linear gradient from 28 to 32% acetonitrile in

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0.1% TFA over a period of 44 min at a flow rate of
1 ml min^{-1} (room temperature). Representative
aliquots of each fraction or pool are removed, taken
to complete dryness by Speed Vac centrifugation, and
5   then re-solubilized in tissue culture medium in
preparation for assay.

Example 7
IL-1 receptor binding assays

10   Radioiodinated IL-1α and IL-1β are obtained
from commercial sources (DuPont NEN Research
Products, Boston, MA) or prepared by chloramine-T
radioiodination of unlabeled peptide.

EL-4 cells (6x10^6/ml; mouse lymphoma cells; ATCC
15   TIB 39, American Type Culture Collection, Rockville,
Maryland) are suspended in binding medium (RPMI 1640
containing 5% fetal calf serum and 25 mM HEPES, pH
7.2). Aliquots (100 μl) are placed in 12 x 75 mm
polypropylene tubes. ^{125}I-labelled IL-1 is added at
20   a concentration of about 1 x10^{-10} M. Incubation is
carried out for 3 hours at 4°C in the presence of
test compound, as described below. Nonspecific
binding is determined by inclusion of 1 x10^{-8} M
unlabeled IL-1. Unbound ^{125}I-IL-1 is separated from
25   cells by centrifugation through 200 μl of a silicone
oil mixture in a Beckman microfuge.

Displacement of binding by test compound is
determined by adding to the above incubation
mixture, varying concentrations of unlabeled test
30   compound ranging from about 10^{-12}-10^{-8} M compound.
IC$_{50}$ and K$_i$ values are calculated for the test
compound according to methods well known in the art
(Kenakin).

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Example 8
Inhibition of Cellular Proliferation

A. Inhibition of IL-1 stimulation of mouse D10
     cell

5   Mouse T helper D10.G4.1 cells (American Type
Culture Collection, Rockland, MD; ATCC TIB 224) were
suspended in culture medium (RPMI 1640 containing 5%
fetal calf serum, 5×10^{-8} M 2-mercaptoethanol, 8
μg/ml gentamicin, 2 mM L-glutamine, and 2.5 μg/ml
10 concanavalin A). Cells were suspended to produce a
cell concentration of about $1-2 \times 10\mu$g/ml. IL-1ra
was added to triplicate cultures, 1 hour prior to
addition of IL-1α or IL-1β to the cultures. The
plates were then incubated and incorporation
15 of [^{3}H]Thymidine into the cells measured according to
standard techniques. In experiments carried out in
support of the present invention, hIL-1α or hIL-1β
was added in increasing concentrations to the assay,
the EC_{50} of hIL-1α was 7 pM and the EC_{50} of hIL-1β was
20 450 pM.   At a concentration of 30 nM, hIL-1ra
completely inhibited stimulation of proliferation by
hIL-1α to a concentration of 100 pM and by hIL-1β to
a concentration of about 100 nM.

        Determination of IC_{50} and K_D values for IL-1
25 receptor antagonists in these assays is accomplished
according to methods known in the art.

B. Inhibition of IL-1 Stimulation of Thymocytes

        Mouse thymocytes are prepared, and the action
30 of IL-1, which produces growth stimulation, is
measured using standard techniques (O'Gara, 1990).
Three to six week old C3H/HeN mice are obtained from
Simonsen Laboratories, Gilroy, California and
sacrificed by standard procedures.   Thymi are
35 immediately removed, separated from adherent
non-thymic tissue, homogenized in Hank's balanced
salt solution (Gibco) using a glass homogenizer, and

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centrifuged at 180 x g for 10 minutes at 15°C.
Following an additional wash in HBSS, the thymocytes
are resuspended in RPMI 1640 tissue culture medium
(Gibco) containing 50µM 2-mercaptoethanol (Fisher),
5   2 mM glutamine (Gibco), 1 mM pyruvate, non-essential
amino acid solution, penicillin (100 U/ml)
streptomycin (100µg/ml) solution, 10% heat
inactivated fetal bovine serum and Phytohemaglutinin
(PHA, Pharmacia, final concentration 10 µg/ml).
10   Cells are cultured in round-bottom 96 well
microtiter tissue culture plates, 6x10^5 cells per
well in a volume of 100 µl. Test compound is
diluted in tissue culture medium and added to the
wells in the presence and absence of IL-1
15   (recombinant human IL-1, R&D Systems catalog #
201-LB, 0.1 ng/ml). Total volume is 150 µl per
well. Plates are incubated for 72 hours (95%
air/5% CO_2, 37°C). During the last four hours of
incubation, tritiated thymidine (Amersham, 49 Ci/mM)
20   is added (0.5 µCi per well). Cells are harvested
onto Whatman 934-AH glass microfiber filters and
counted in a Beckman LS 6000 scintillation counter.
Results are expressed as counts per minute well.
Cytotoxicity is measured in these assays using the
25   MTT reduction assay, as described below.

        Untreated cells show minimal DNA synthesis
(thymidine incorporation 80 cpm/well). PHA alone
produces a 2-3 fold stimulation of cell
proliferation. Treatment with 0.1 ng/ml IL-1 in the
30   presence of PHA results in 60 fold stimulation.
Addition of the IL-1ra results in a dose dependent
inhibition of IL-1 stimulation.

                            Example 9
35   IL-6 Bioassay
        Mice were given IL-1 are injected with IL-1ra
(5 mg/kg, subcutaneously,(s.c.)) or test receptor

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antagonist. Mice are immediately given IL-1α (5
μg/kg s.c.). Blood samples from the mice are taken
3 hours later.

5   Serum levels of IL-6 are determined using a
modification of the B9 hybridoma cell assay
described by Aarden et al. and incorporated herein
by reference. B9 cells are treated with twofold
serial dilutions of the test sera in 96-well
microtiter plates. After a 3-day incubation at 37°
10   in a humidified atmosphere of 5% CO_2 and 95% air, the
wells are pulsed with 0.5 μCi of [^{3}H]thymidine
([^{3}H]TdR) and incubated for an additional 18 hours.
The cells are then harvested onto glass fiber
filters and the level of [^{3}H]TdR incorporation
15   determined in a liquid scintillation counter.   IL-6
units are defined as the inverse of the serum
dilution   that   produces   half-maximal   [^{3}H]TdR
incorporation compared with a reference standard.

20   Example 10
                  Prevention of Ovulation
       Female rats were administered PMSG (25 I.U.),
then anesthetized with pentobarbital. One horn of
the uterus was exposed, and 1 μg of IL-1ra was
25   injected unilaterally directly into the ovarian
bursa.   Animals   were   then   stimulated   by
administration of hCG (25 I.U.). Animals were later
examined   for   presence   of   ova.   Using   the
contralateral uninjected ovary as control, a 40%
30   reduction in ovulation was observed after IL-1ra
treatment.
       While the invention has been described with
reference to specific methods and embodiments, it
will be appreciated that various modifications and
35   changes may be made without departing from the
invention.

50

IT IS CLAIMED:

1.   A composition for use in preventing
ovulation and implantation of an embryo in a uterus
5   of a mammalian subject, comprising an IL-1 receptor
antagonist.

2.   The composition of claim 1, wherein the
interleukin-1 receptor antagonist is a peptide.
10

3.   The composition of claim 2, wherein said
interleukin receptor antagonist is selected from the
group consisting of IL-1ra (SEQ ID NO: 2) and icIL-
1ra (SEQ ID NO: 4).
15

4.   The composition of claim 3, wherein said
interleukin receptor antagonist is IL-1ra (SEQ ID
NO: 2).

20   5.   The composition of claim 1, wherein said
compound is selected from a combinatorial library of
compounds, by the steps of:
measuring (i) agonist activity, and (ii)
antagonist activity of a library of compounds, in a
25   functional IL-1 receptor assay, and
selecting a specific compound in the library if
it has (i) substantially no agonist activity, and
(ii) substantial antagonist activity.

30   6.   A contraceptive device for use in
preventing ovulation and implantation of an embryo
or in dislodging an embryo in a mammalian uterus,
comprising
a vaginal insert or a cervical cap, and
35   compound release means in said insert or cap
for releasing an IL-1 receptor antagonist compound

at a dose effective to prevent or disrupt implantation of an embryo in the uterus.

5   7.   The device of claim 6, wherein the interleukin-1 receptor antagonist is a peptide.

8.   The device of claim 7, wherein said interleukin receptor antagonist is selected from the group consisting of IL-1ra (SEQ ID NO: 2) and icIL-
10   1ra (SEQ ID NO: 4), and said compound release means is effective to produce a concentration of said peptide at the uterus of about 1-50 μg/ml.

9.   The device of claim 6, wherein the said
15   interleukin receptor antagonist is selected from a combinatorial library of compounds, by the steps of:
    measuring (i) agonist activity, and (ii) antagonist activity of a library of compounds, in a functional IL-1 receptor assay, and
20   selecting a specific compound in the library if it has (i) substantially no agonist activity, and (ii) substantial antagonist activity.

10. A method of identifying a compound for use
25   in preventing ovulation and implantation of an embryo or in dislodging an embryo in a mammalian uterus, comprising
    measuring (i) agonist activity, and (ii) antagonist activity of a library of compounds, in a
30   functional IL-1 receptor assay, and
    selecting a specific compound in the library if it has (i) substantially no agonist activity, and (ii) substantial antagonist activity.

35   11.   The method of claim 10, wherein said measuring further includes measuring the binding affinity of the compound in an IL-1R tI IL-1 ligand

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competitive displacement binding assay, and said
selecting further includes selecting the compound if
its affinity in such binding assay is at least
within two orders of magnitude of the binding
5   affinity of an IL-1 receptor antagonist selected
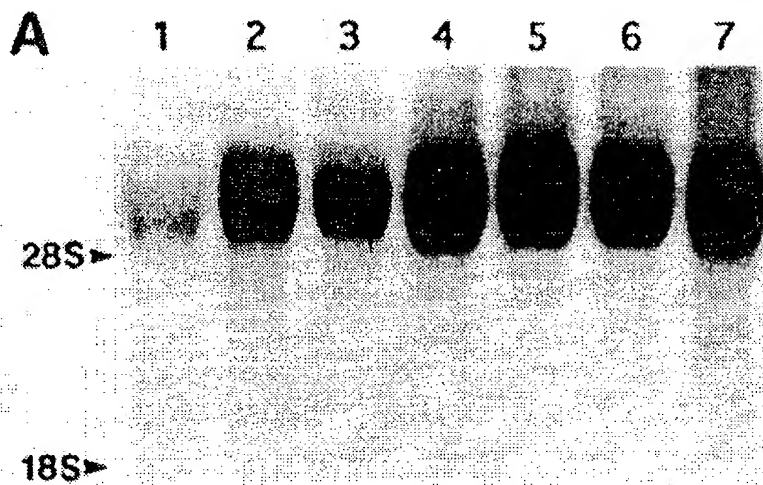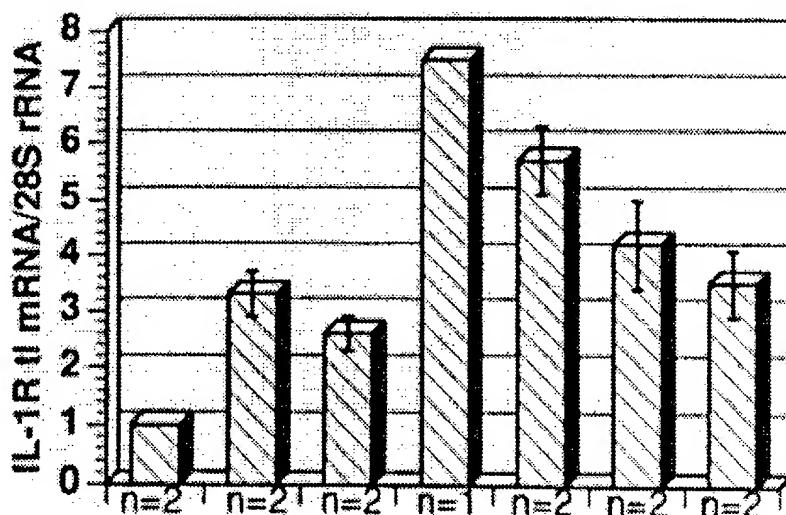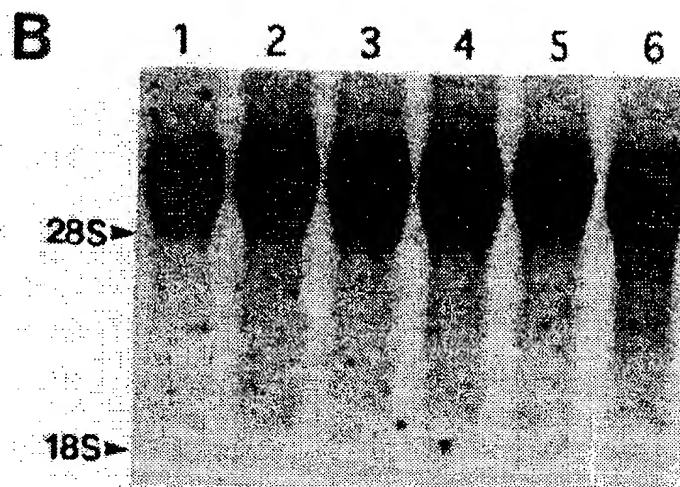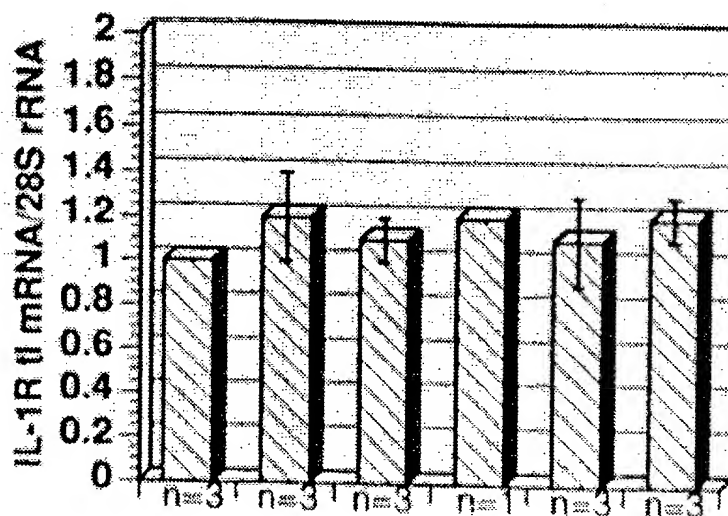from the group consisting of IL-1ra and icIL-1ra.

Fig. 1A

Fig. 1B

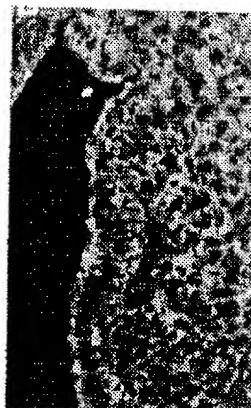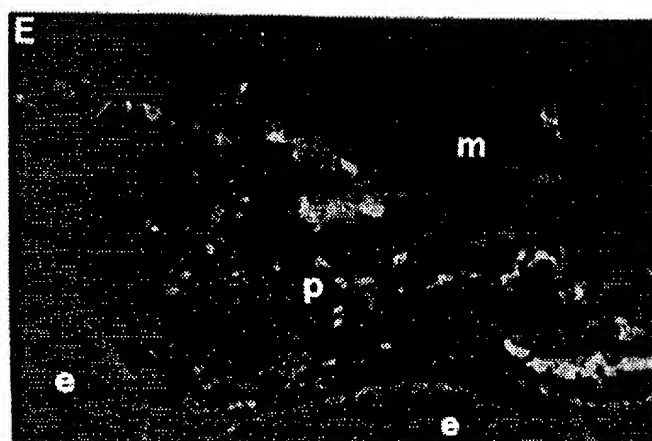Fig. 2A

Fig. 2B
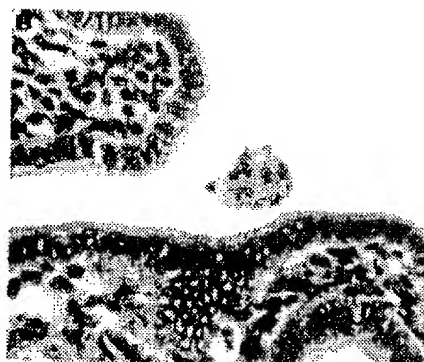
Fig. 3A   Fig. 3B   Fig. 3C

Fig. 3D

Fig. 3E

Fig. 4A   Fig. 4B

Fig. 4C   Fig. 4D

Fig. 4E   Fig. 4F

Fig. 5

```
  1 GAATTCCGGGCTGCAGTCACAGA                                                      23

    ATG GAA ATC TGC AGA GGC CTC CGC AGT CAC CTA ATC ACT CTC CTC CTC TTC CTG TTC CAT    83
     M   E   I   C   R   G   L   R   S   H   L   I   T   L   L   L   F   L   F   H

    TCA GAG ACG ATC TGC CGA CCC TCT GGG AGA AAA TCC AGC AAG ATG CAA GCC TTC AGA ATC   143
     S   E   T   I   C   R   P   S   G   R   K   S   S   K   M   Q   A   F   R   I(15)

    TGG GAT GTT AAC CAG AAG ACC TTC TAT CTG AGG AAC AAC CAA CTA GTT GCT GGA TAC TTG   203
     W   D   V   N   Q   K   T   F   Y   L   R   N   N   Q   L   V   A   G   Y   L(35)

    CAA GGA CCA AAT GTC AAT TTA GAA GAA AAG ATA GAT GTG GTA CCC ATT GAG CCT CAT GCT   263
     Q   G   P   N   V   N   L   E   E   K   I   D   V   V   P   I   E   P   H   A(55)

    CTG TTC TTG GGA ATC CAT GGG GGG AAG ATG TGC CTG TCC GTC GTC AAG TCT GGT GAT GAG   323
     L   F   L   G   I   H   G   G   K   M   C   L   S   V   V   K   S   G   D   E(75)

    ACC AGA CTC CAG CTG GAG GCA GTT AAC ATC ACT GAC CTG AGC GAG AAC AGA AAG CAG GAC   383
     T   R   L   Q   L   E   A   V   N   I   T   D   L   S   E   N   R   K   Q   D(95)

    AAG CGC TTC GCC TTC ATC CGC TCA GAC AGT GGC CCC ACC AGT TTT GAG TCT GCC GCC GCC   443
     K   R   F   A   F   I   R   S   D   S   G   P   T   S   F   E   S   A   A   A(115)

    TGC CCC GGT GCC TGG CTC TGC ACA GCG ATG GAA GCT GAC CAG CCC GTC AGC CTC ACC AAT   503
     C   P   G   A   W   L   C   T   A   M   E   A   D   Q   P   V   S   L   T   N(135)

    ATG CCT GAC GAA GGC GTC ATG GTC ACC AAA TTC TAC TTC CAG GAG GAC GAG TAG           557
     M   P   D   E   G   V   M   V   T   K   F   Y   F   Q   E   D   E(152) *
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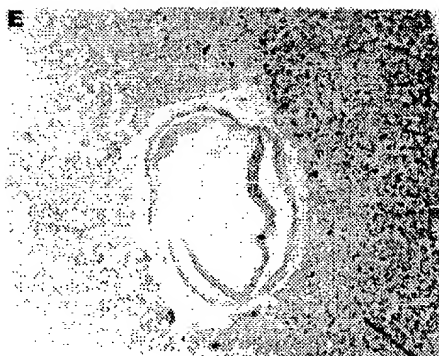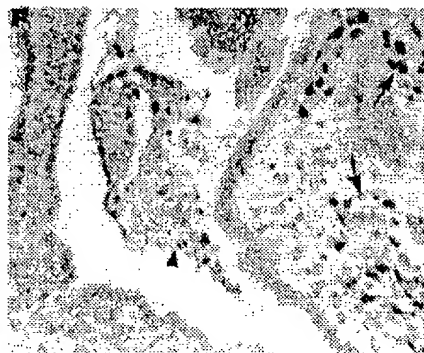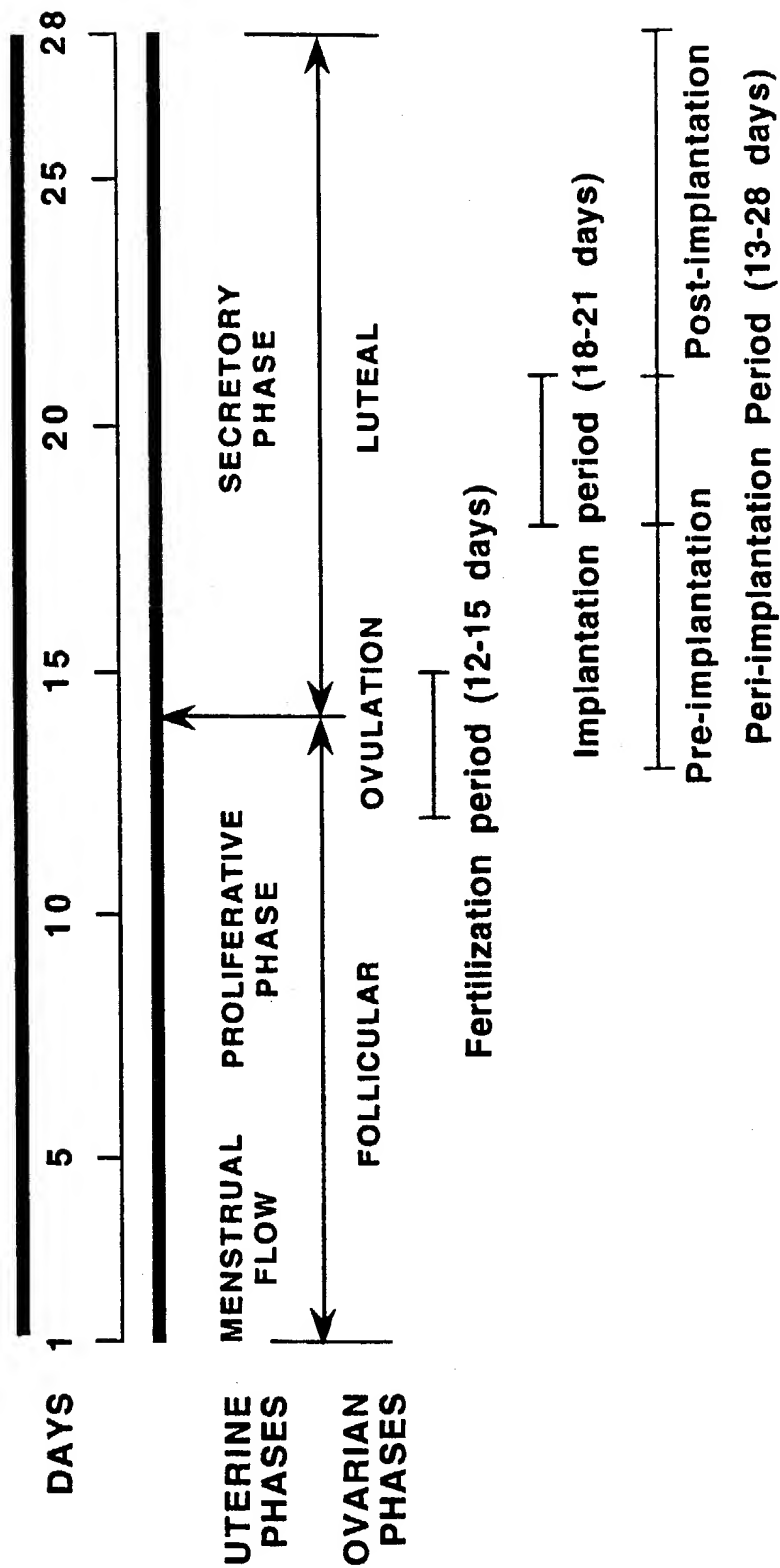
Fig. 6

Fig. 7

1   AGCTCCACCCTGGGAGGACTGTGGCCCCAGGTACTGCCCGGGGTGCTACTTTATGGGCAGCAGCTCAGTTGAGTTAGAGTCTGGAGAAGACCTCCTGTCCTATGAGGCCCTCC
    GM406   GM397

121 CCATGGCTTTAG 132
    M A L E

133 AGACGGATCTGCCGACCCTCTGGGAGAAATCCAGCAAGATGCAAGCCTTCAGAATCTGGGATGTTAACCAGAAGACCTTCTATCTGAGGAACAACCACTAGTTGCTGGATACTTGCAAG
    T I C * R P S G R K S S K M Q A F R I W D V N Q K T F Y L R N N Q L V A G Y L Q G
    GM341   GM343   GM415

253 GACCAAATGTCAATTTAGAGAAGAAAGATAGATGTGTACCCATTGAGCCTCATGCTCTGTTCTTGGAATCCATGGAGGCAAGATGCCTGTCTCGTGTCAAGTCTGGTGATGACACCA
    P N V N L E K I D V V P I E P H A L F L G I H G G K M C L S C V K S G D E T R
    GM420   GM368

373 GACTCCAGCTGGAGGCAGTTAACATCACTGACCTGAGCGAGAACAGAAAGCAGGACAAGCGGTTCGCCTTCATCCGCTCAGACAGTGGCCCCCCACCAGTTTGAGTCTGCCCCCTGCC
    L Q L E A V N I T D L S E N R K Q D K R F A F I R S D S G P T T S F E S A A C P

493 CCGGTTGGTTCCTCTGCACAGCGATGGAAGCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGGCTCATGGTCACCAAATTCTACTTCCAGGAGGACGAGTAG
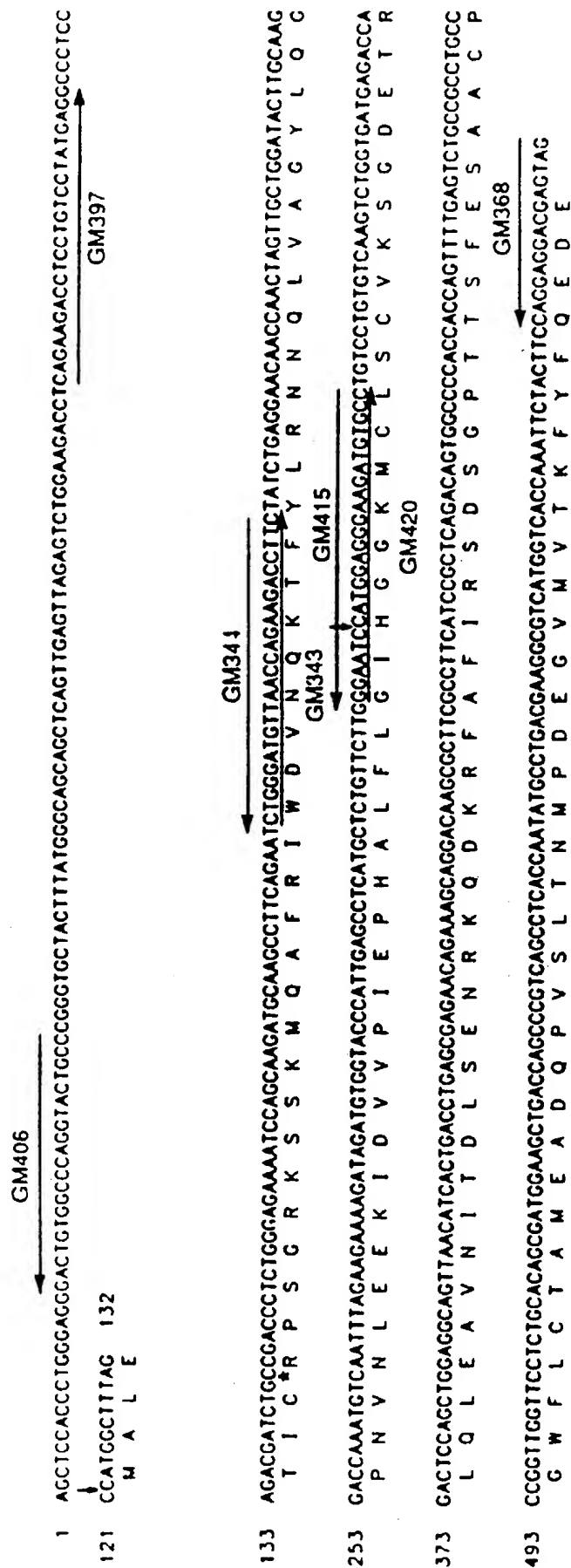   G W F L C T A M E A D Q P V S L T N M P D E G V M V T K F Y F Q E D E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/11588

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 38/20
US CL : 514/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, EMBASE, MEDLINE, WPIDS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proc. Natl. Acad. Sci. USA, Volume 88, issued May 1991, S. Haskill et al., "cDNA cloning of an Intracellular Form of the Human Interleukin 1 Receptor Antagonist associated with Epithelium", pages 3681-3685, see whole document.	1-2, 5-7, 9-10
Y	Biology of Reproduction, Volume 14, issued 1976, I. Aref et al., "Utero-Oviductal Motility, Egg Transport and Implantation in Prostaglandin Treated Rabbits", pages 658-664, see whole document.	1-2, 5-7, 9-10
Y	Am. J. Obstet. Gynecol., Volume 167, No. 4, Part 1, issued October 1992, R. Romero et al., "The natural interleukin-1 receptor antagonist in term and preterm parturition", pages 863-872, see whole document.	1-2, 5-7, 9-10

[X] Further documents are listed in the continuation of Box C.	[ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 DECEMBER 1994	FEB 1 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer C. SAYALA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,116,619 (GRECO ET AL.) 26 May 1992, see whole document.	6-7, 9
Y	US, A, 4,961,436 (KOCH) 09 October 1990, see whole document.	6-7, 9
Y	Nature, Volume 343, issued 25 January 1990, C. Hannum et al., "Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor", pages 336-340, see whole document.	5, 9-10
Y	J. Exp. Med., Volume 173, issued April 1991, K. McIntyre et al., "Inhibition of Interleukin 1 (IL-1) Binding and Bioactivity In Vitro Modulation of Acute Inflammation In Vivo by IL-1 Receptor Antagonist and Anti-IL-1 Receptor Monoclonal Antibody", pages 931-939, see whole document.	5, 9-10
Y	J. Immunology, Volume 138, No. 9, issued 01 May 1987, S. Mizel et al., "The interleukin 1 receptor. Dynamics of Interleukin 1 Binding and Internalization in T Cells and Fibroblasts", pages 2906-2912, see whole document.	5, 9-10